PB80-220056

Effect of Cadmium on the Root and Nodule Ultrastructure of 'Alnus rubra'

(U.S.) Corvallis Environmental Research Lab., OR

1980

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-80-032	2. JOURNAL ARTICLE	3. RECIPIENT'S ACCESSION NO. PB80 220056
4. TITLE AND SUBTITLE: Effect of Cadmium on the Root and Nodule Ultrastructure of Alnus rubra		5. REPORT DATE: 1980 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Carlos Wickliff		8. PERFORMING ORGANIZATION REPORT NO
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Research Laboratory-Corvallis Office of Research and Development U.S. Environmental Protection Agency Corvallis, Oregon 97330		10. PROGRAM ELEMENT NO 11. CONTRACT/GRANT NO
12. SPONSORING AGENCY NAME AND ADDRESS: SAME		13. TYPE OF REPORT AND PERIOD COVERED: Journal article 14. SPONSORING AGENCY CODE: 600/02

15. SUPPLEMENTARY NOTES

Published in Environmental Pollution (Series A) 21:1980, pp. 287-306.

16. ABSTRACT

Alnus rubra (Bong.) was grown for three months in a greenhouse and given 0, 6, 12, 31 and 62 µg cadmium/liter of nitrogen-free nutrient solution for 11 weeks. Segments of nodules and secondary roots were collected from plants grown at the different Cd levels. Thin sections were made for transmission electron microscopy and the ultrastructure of root and nodule cells was then investigated. Endophyte resorption advanced in the nodules and the number of starch grains in root xylem parenchyma cells tripled as the cadmium concentrations increased from 12 to 62 µg/liter of nutrient solution. Nucleoli increased in prominence in the nuclei of the root xylem parenchyma cells as the cadmium concentration in the nutrient solution was raised from 12 to 62 µg/liter.

Observations of root and nodule cell ultrastructure of Alnus rubra suggest that cadmium exerts its toxic effect in roots and nodules by decreasing the number of endophyte structures capable of fixing nitrogen and reducing the rate of respiration and growth by host plant cells.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: Release to Public	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS (This page): Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

PB80-220056

EPA-600/J-80-032
JOURNAL ARTICLE

a reprint from

Environmental Pollution

an International Journal

Edited by

KENNETH MELLANBY

Published by

APPLIED SCIENCE PUBLISHERS LTD

Ripple Road, Barking, Essex, England

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

Environmental Pollution (Series A) 21 (1980) 287–306

EFFECT OF CADMIUM ON THE ROOT AND NODULE ULTRASTRUCTURE OF *ALNUS RUBRA*

CARLOS WICKLIFF

Environmental Protection Agency, Terrestrial Systems Division, 200 S.W. 35th Street, Corvallis, Oregon 97330, USA

&

H. J. EVANS

Laboratory for Nitrogen Fixation Research, Oregon State University, Corvallis, Oregon 97331, USA

ABSTRACT

Alnus rubra (*Bong.*) *was grown for three months in a greenhouse and given 0, 6, 12, 31 and 62 μg cadmium/litre of nitrogen-free nutrient solution for 11 weeks. Segments of nodules and secondary roots were collected from plants grown at the different Cd levels. Thin sections were made for transmission electron microscopy and the ultrastructure of root and nodule cells was then investigated. Endophyte resorption advanced in the nodules and the number of starch grains in root xylem parenchyma cells tripled as the cadmium concentration increased from 12 to 62 μg/litre of nutrient solution. Nucleoli increased in prominence in the nuclei of the root xylem parenchyma cells as the cadmium concentration in the nutrient solution was raised from 12 to 62 μg/litre.*

Observations of root and nodule cell ultrastructure of Alnus rubra *suggest that cadmium exerts its toxic effect in roots and nodules by decreasing the number of endophyte structures capable of fixing nitrogen and reducing the rate of respiration and growth by host plant cells.*

INTRODUCTION

Alnus rubra (Bong.), known for its nitrogen contribution to forest soils (Tarrant, 1961; Tarrant & Miller, 1963) is a hardwood tree species used commercially for furniture and charcoal production. This species is widely distributed in the Douglas fir *Pseudotsuga menziesii* region of the Pacific Northwest. It is a non-leguminous angiosperm that causes increased growth of dominant Douglas fir, when mixed with the fir, by improving the fertility of the forest soil (Tarrant, 1961).

Environ. Pollut. Ser. A. 0143-1471/80/0021-0287. Applied Science Publishers Ltd, England, 1980
Printed in Great Britain

Greenhouse studies have shown that cadmium, an environmental pollutant, inhibits biological nitrogen fixation by soybeans (Huang *et al.*, 1974). The prevalence of cadmium pollution (Allaway, 1968; Special Studies Staff, 1975) and the importance of *Alnus* species in soil fertility were the bases for this investigation, which was designed to obtain data on the extent to which cadmium would alter the root nodule ultrastructure of *Alnus rubra*.

MATERIALS AND METHODS

Seeds of *Alnus rubra* were obtained from an *Alnus* stand along the Oregon coast near Yachats, Oregon, and stored in a refrigeration room at 0° to 4°C for six months. They were then removed from storage, inoculated with a suspension of crushed nodules from mature trees and germinated in moist vermiculite. The seedlings were irrigated twice weekly with nitrogen-free nutrient solution. Mature nodules were present on the roots of each plant after three months' growth in the vermiculite. Seedlings that were uniform in appearance were transplanted into sand culture to which nitrogen-free nutrient solutions containing cadmium as cadmium chloride at concentrations of 0, 6, 12, 31 and 62 μg/litre were applied. These plants were irrigated daily with 3·2 litres of nitrogen-free nutrient solution which was collected in receiving containers and re-used for 6 days each week. On day 7 the plants were excessively irrigated with distilled water to prevent salt accumulation in the sand. On the same day the old nutrient solution was discarded and replaced with fresh solution. Cadmium was applied to test plants for 11 weeks and six replicate plant cultures with two plants per culture were utilised for each cadmium treatment. Top and root specimens of *Alnus rubra* seedlings were obtained from the *Alnus* stand for measurement of cadmium concentrations in plants from the forest.

Cadmium was extracted from 50 g samples of sand used to culture *A. rubra* seedlings by stirring for 45 min with deionised water and with 0·01N HCl solution before and after water extraction. The suspensions were allowed to settle, after which the supernatant solutions were decanted, filtered and analysed for cadmium by atomic absorption spectrophotometry.

Statistically significant differences between means shown in Tables 1 and 2 were determined by analysis of variance and the least significant difference (LSD) test. Differences between means are statistically significant at the 5% level of statistical significance if they are equal to, or greater than, the LSD (5%) value computed for the parameter.

Segments of nodules and secondary roots of *Alnus rubra* were removed from a central location in the root systems of plants grown 11 weeks at each cadmium concentration. Nitrogenase activity was measured in the remaining nodules by the acetylene reduction method (Fishbeck *et al.*, 1973). The plants were then separated into leaves, stems, roots and nodules, dried for 24 h at 70°C and weighed. All dry

TABLE I

EFFECT OF CADMIUM ON NITROGENASE ACTIVITY, GROWTH AND CADMIUM UPTAKE BY *Alnus rubra*

Cd conc. in nutrient solution (μg/litre)	*Nitrogenase activity*[a]	*Number nodules per plant*	*Dry weight (g)*			*Cadmium concentration (μg/g)*			
			Leaves	*Stem*	*Root*	*Leaves*	*Stem*	*Root*	*Nodules*
0	54·4	109	3·49	1·80	3·74	0·0	0·0	0·05	0·01
6	50·9	136	4·41	2·40	3·81	0·6	1·0	9·3	1·8
12	43·9	137	3·87	1·64	3·70	1·6	1·8	26·8	5·0
31	38·0	179	4·89	2·07	2·10	2·3	3·6	60·9	19·9
62	26·1	169	3·45	1·06	1·34	3·1	18·8	129·8	47·9
LSD (5%)[b]	22·2	60	3·11	1·34	1·82	0·6	13·9	16·8	—

[a] Nitrogenase activity in the nodules of *Alnus rubra* was estimated by the acetylene reduction method (Fishbeck *et al.*, 1973) and is expressed as the rate of nitrogenase-dependent reduction of acetylene to ethylene. The values given are micromoles of ethylene formed per gramme of dry nodule per hour.

[b] Least significant difference at the 5% level of statistical significance (see 'Materials and Methods').

TABLE 2
CADMIUM CONCENTRATION IN THE SAND USED TO CULTURE *Alnus rubra*

Cadmium concentration in nutrient solution (μg/litre)[a]	*Cadmium concentration in sand culture (μg/g dry sand)*		
	Water extraction	*0·01 N HCl extraction*	*0·01 N HCl extraction after extraction by water*
0	0·00	0·42	0·41
6	0·01	0·44	0·42
12	0·02	0·48	0·44
31	0·07	0·52	0·48
62	0·14	0·63	0·57
LSD (5%)[b]	0·02	0·20	0·10

[a] Before addition to sand culture.
[b] Least significant difference at the 5% level of statistical significance.

plant material was ground in a Wiley mill and analysed for cadmium by nitric–perchloric acid digestion followed by atomic absorption spectrometry. Segments of nodules and roots designated for electron microscopy were thinly sectioned with a razor blade and fixed for 6 h at room temperature in 5% glutaraldehyde solution in 0·1 M Sorensen's monobasic–dibasic phosphate buffer, pH 7 (17·2 g KH_2PO_4 and 17·8 g K_2HPO_4 per litre of distilled water). The segments were then extensively washed in the buffer alone and treated with 0·1% OsO_4 until blackening occurred. The segments were dehydrated by washing for 15 min in 20, 50 and 70% acetone in distilled water followed by 15 min in 100% acetone. Uranyl acetate in the 70% acetone was used to stain the segments. After dehydration the segments were placed in a mixture of one part acetone to two parts plastic (53 ml dodecenyl succinic anhydride (DDSA), 35 ml Araldite 6005 and 12 ml Epon 812 resin). The segments were allowed to stand for 24 h in the plastic–acetone mixture, after which they were embedded in plastic containing one drop of the catalyst (benzyldimethylamine or tridimethylaminomethyl phenol) per millilitre of plastic. The blocks formed were cured for 24 h at 60–70 °C, trimmed with a clean razor blade, and sectioned to a thickness of 900 to 1500 Å with a glass knife. The sections were mounted on copper grids coated with Formvar (0·5% polyvinyl formal in ethylene dichloride) and then observed with a Phillips EM 300 or a Phillips EM 200 transmission electron microscope. Magnification of the specimens is within 5% of those given in the accompanying figures.

RESULTS

Nodule ultrastructure

The hyphal and vesicular forms of the endophyte from control plants are surrounded by a cell wall and enclosed in a capsular material surrounded by the plasmalemma (Figs 1 and 2). Fine threads of what appear to be nucleic acid

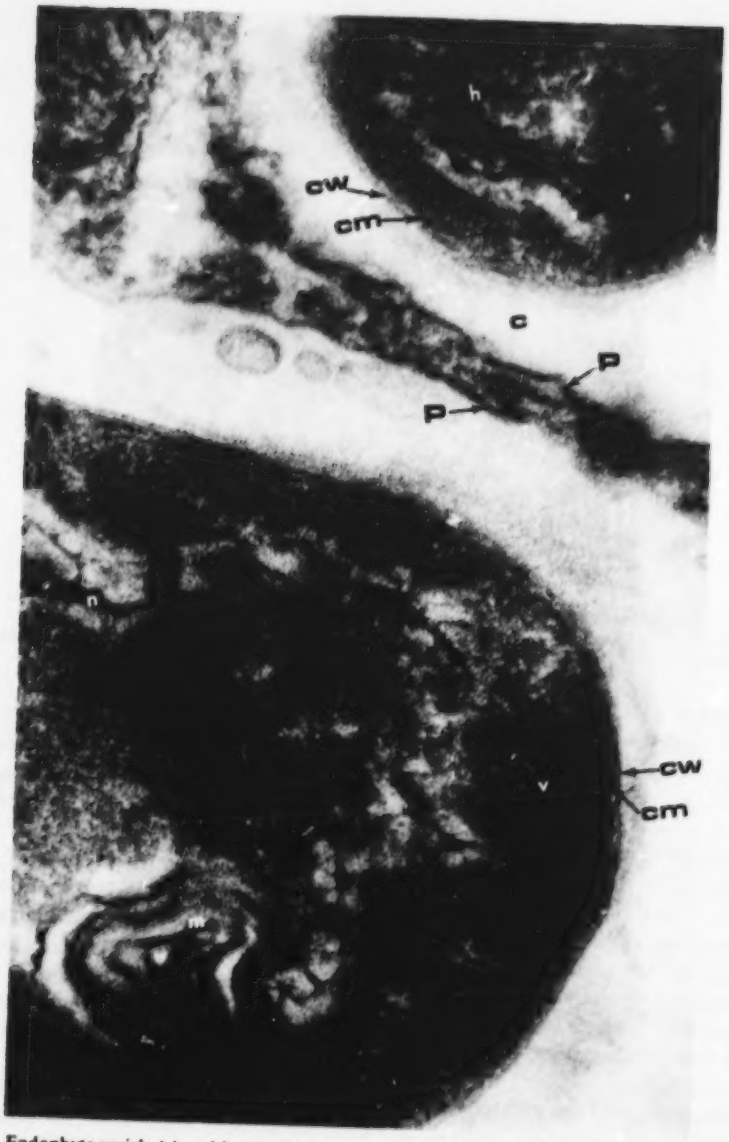

Fig. 1. Endophyte vesicle (v) and hypha (h) from the nodules of a control plant given no cadmium. Magnification 34,000 ×. (Capsular material c, endophyte cell membrane cm, endophyte cell wall cw, mesosome m, plasmalemma P.)

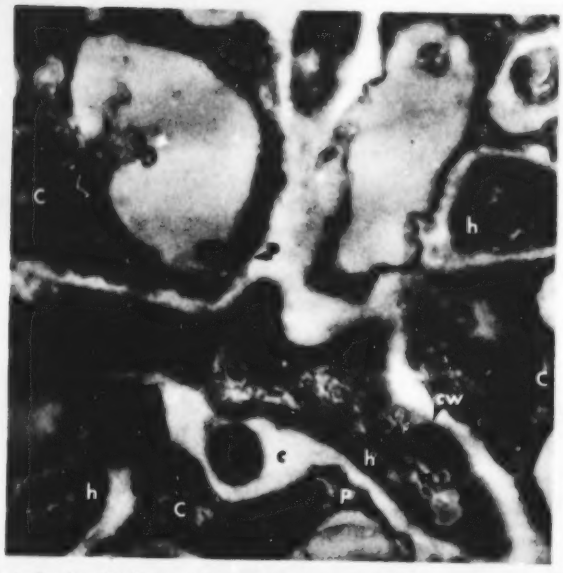

Fig. 2. Endophyte hyphae (h) from the nodule of a control plant given no cadmium. One hypha was sectioned longitudinally. Note the outgrowths from this hypha. Magnification 8,000×. (Host cytoplasm C, capsular material c, endophyte cell wall cw, endophyte nuclear material n, plasmalemma P.)

polymers attached to dense bodies were seen inside the endophyte (Fig. 1). The hyphae are divided transversely by septa which are also seen in some vesicles (Fig. 3). In addition, longitudinal sections of hyphae contained circular structures not previously described (Fig. 4). (The structures were later identified as glycogen by M. Lalonde and J. G. Torrey at the 1978 Steenbock-Kettering International Symposium on nitrogen fixation, University of Wisconsin–Madison).

The ultrastructure of the endophyte and host cells from the nodules of plants given 6 and 12 μg Cd/litre of nutrient solution (Figs 5–7) did not differ from that of the control. Numerous septa were seen within vesicles from plants given 31 μg Cd/litre (Figs 8–10). It was also observed that the vesicles and host cell cytoplasm appeared to be collapsing (Fig. 10). Septa were numerous in the endophyte vesicles of plants treated with 31 and 62 μg Cd/litre (Figs 8 and 11); however, mesosomes and glycogen could not be distinguished at these concentrations.

Resorption of the endophyte is characterised by progressive lysis of endophyte cytoplasm and occurs naturally at the base of the nodules (Lalonde & Knowles, 1975). However, this phenomenon was observed in the centre of nodules collected from plants treated with 31 and 62 μg Cd/litre of nutrient solution (Figs 10, 12 and 13).

Fig. 3.

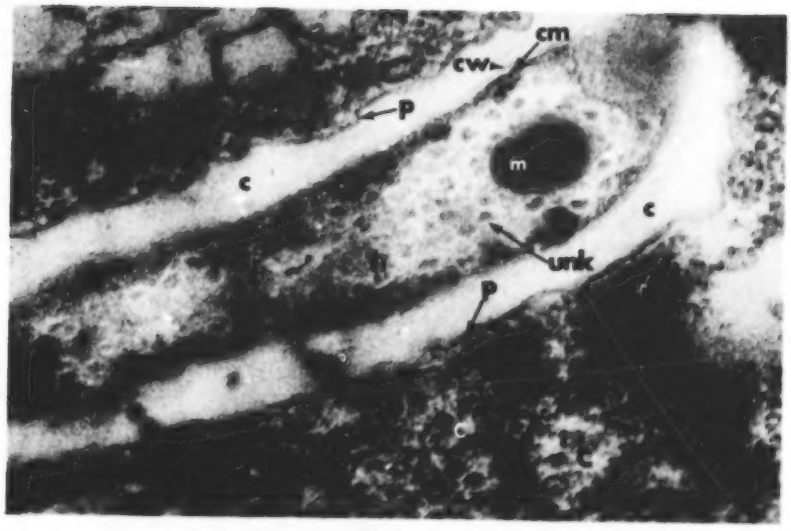

Fig. 4.

Figs. 3 and 4. Longitudinal sections of hyphae (h) from the nodules of control plants given no cadmium. Capsular material (c) and host cell plasmalemma (P) surround and enclose the hyphae. Note the continuity of the endophyte cell wall (cw) and cytoplasmic membrane (cm) in septum (s) formation. Magnification 8,000 × (Fig. 3) and 34,000 × (Fig. 4). (Amyloplast A, host cytoplasm C, mesosome m, unknown unk.)

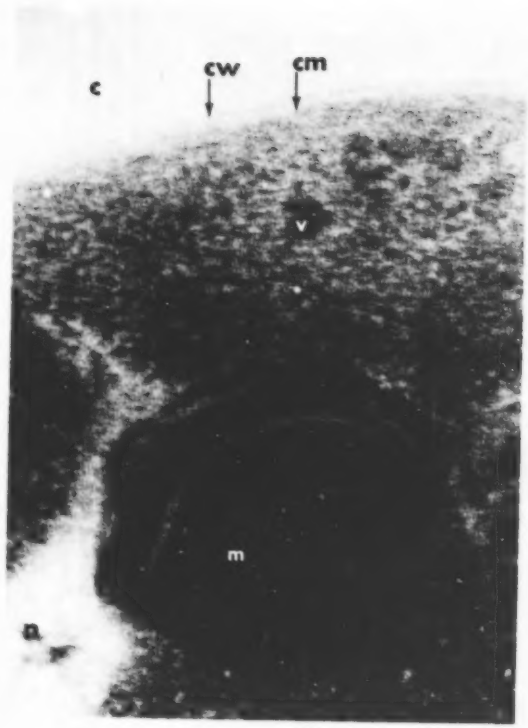

Fig. 5.

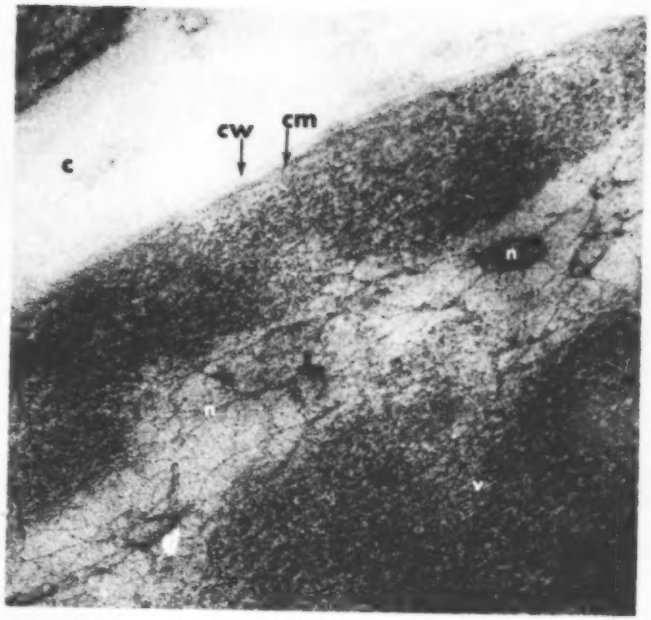

Fig. 6.

Figs. 5 and 6. Endophyte vesicles (v) from the nodules of plants treated with 6 μg Cd/litre of nutrient solution. The ultrastructure of the vesicles did not differ from that of control plants given no cadmium. Magnification 34,000 × . (Capsular material c, endophyte cell membrane cm, endophyte cell wall cw, mesosome m, endophyte nuclear material n.)

Fig. 7. Endophyte hyphae (h) from the nodule of a plant treated with 6 μg Cd/litre of nutrient solution. The ultrastructure of the nyphae did not differ from that of control plants given no cadmium. Longitudinal section shows branching of the hypha. Magnification 8,000 ×. (Host cytoplasm C, capsular material c, endophyte cell wall cw, mesosome m, endophyte nuclear material n, plasmalemma P, septum s.)

The result is collapse of endophyte structure culminating in 'ghost' vesicles and hyphae which lack protoplasts (Figs 12 and 13). The early and later stages of endophyte resorption were observed in specimens taken from the nodules of plants treated with 31 and 62 μg Cd/litre of nutrient solution, respectively (Figs 10, 12 and 13). Resorption was not seen at cadmium concentrations of 0 to 12 μg Cd/litre but was observed to be nearly complete in specimens from plants treated with 62 μg Cd/litre (Figs 12 and 13).

Root ultrastructure

Xylem parenchyma cells were observed in secondary roots collected from plants treated with cadmium. Neither starch grains nor nucleoli were identified in the nuclei from root xylem parenchyma cells of control plants although more than a hundred observations were made (Fig. 14). The cells of plants treated with 6 μg Cd/litre of nutrient solution did not differ from those of the control plants, but nucleoli and starch grains appeared in a quarter of the cells observed when the cadmium concentration was raised to 12 μg/litre (Fig. 15). The cells from plants given 31 μg Cd/litre of nutrient solution were similar to those from plants given 12 μg/litre. Therefore, photographs of those cells are not presented. Nucleoli were observed in nearly every xylem parenchyma cell taken from roots of plants treated with 62 μg Cd/litre. The nucleus and endoplasmic reticulum were densely stained and a prominent nucleolus consistently appeared in the nucleus at this concentration

Fig. 8. Endophyte vesicle (v) from the nodule of a plant treated with 31 μg Cd/litre of nutrient solution. There were more septa(s) and the nuclear material (n) was more condensed at this Cd concentration. Magnification 25,000 ×. (Capsular material c.)

Fig. 9. Dense endophyte vesicle (dv) from the nodule of a plant treated with 31 μg Cd/litre of nutrient solution. Dense vesicles were seen more frequently in the nodules of plants grown at this Cd concentration. Magnification 8,000 ×. (Capsular material c, host cell wall CW, endophyte nuclear material n, plasmalemma P, septum s.)

Fig. 10. Partially resorbed vesicle (rv) from the nodule of a plant treated with 31 μg Cd/litre of nutrient solution. The endophyte cytoplasm of this vesicle has undergone resorption and the cell is collapsing. Magnification 8,000 ×. (Capsular material c, endophyte cell wall cw, septum s.)

Fig. 11. Endophyte vesicle (v) from the nodule of a plant treated with 62 μg Cd/litre of nutrient solution. This vesicle is in the same stage of resorption as that in Fig. 8 although the Cd concentration was greater. Magnification 25,000 ×. (Endophyte cell membrane cm, endophyte cell wall cw, endophyte nuclear material n, septum s.)

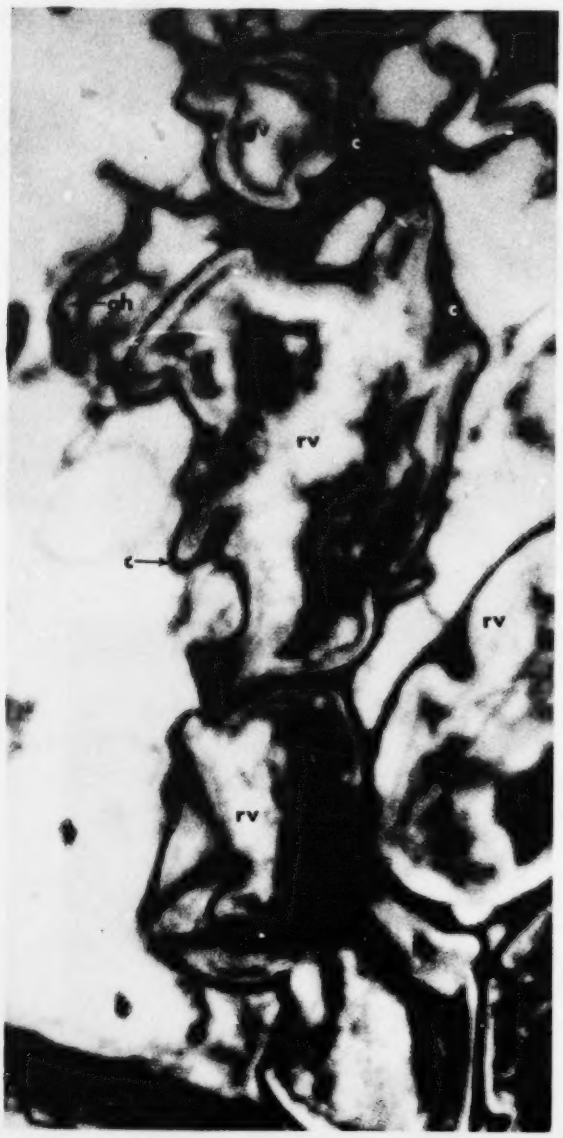

Fig. 12. Endophyte hyphae and vesicles from the nodules of a plant treated with 62 μg Cd/litre of nutrient solution. The hyphae have been totally resorbed to form ghost hyphae (gh). The vesicles have been either partially (rv), or totally resorbed to form ghost vesicles (gv). Both structures have retained the capsular material (c) around them. Magnification 8,000 ×.

Fig. 13. (See caption for Fig. 12.)

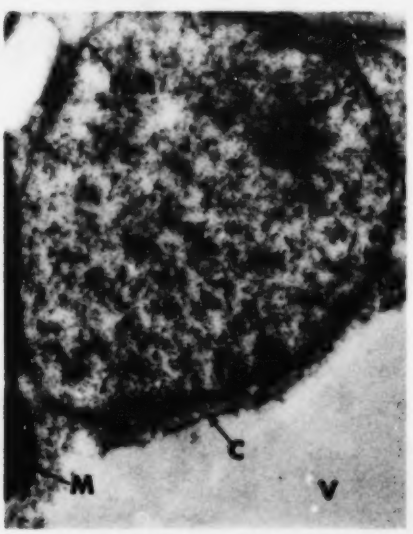

Fig. 14. Nucleus (N) of root xylem parenchyma cells from control plant which received no cadmium. A nucleolus could not be seen despite repeated observations. Magnification 8,000 × . (Host cytoplasm C, mitochondrion M, nuclear membrane NM, nucleopore NP, vacuole V.)

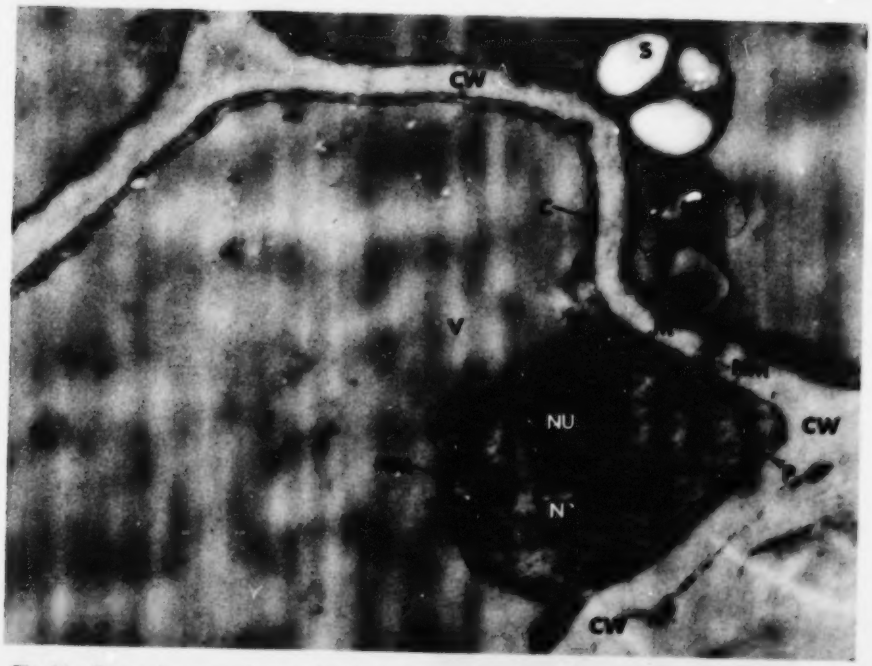

Fig. 15. Root xylem parenchyma cells from a plant which was treated with 12 μg Cd/litre of nutrient solution. Starch grains (S) were observed in the cytoplasm (C) and nucleoli (NU) were seen in several nuclei (N) at this Cd concentration. Magnification 4,750 ×. (Host cell wall CW, mitochondrion M, nuclear membrane NM, plasmalemma P, vacuole V.)

(Fig. 16). Starch grains were more numerous in cells treated with 62 μg Cd/litre and appeared in nearly every cell observed (Fig. 17).

Nitrogenase activity, plant growth and cadmium absorption

Nitrogenase activity (nitrogenase-dependent acetylene reduction) decreased as the cadmium concentration increased in the nutrient solution (Table 1). The number of nodules per plant increased as the cadmium concentration increased, although the dry weights of the other plant organs did not. Root and stem dry weights were decreased significantly below those of control plants when the cadmium concentration was 62 μg/litre (Table 1). Cadmium concentration increased in all plant organs as that in the nutrient solution increased. A high negative correlation ($r = -0{\cdot}96$) was observed between nitrogenase activity and cadmium concentration in the nodules. Cadmium was found in *Alnus rubra* plants from the *Alnus* stand at a mean concentration of 11 μg/g dry weight in both top and root specimens.

Fig. 16. Nucleus (N) of a root xylem paranchyma cell from a plant treated with 62 μg Cd/litre of nutrient solution. Note the prominent nucleolus (NU). Magnification 8,000×. (Host cytoplasm C, host cell wall CW, vacuole V.)

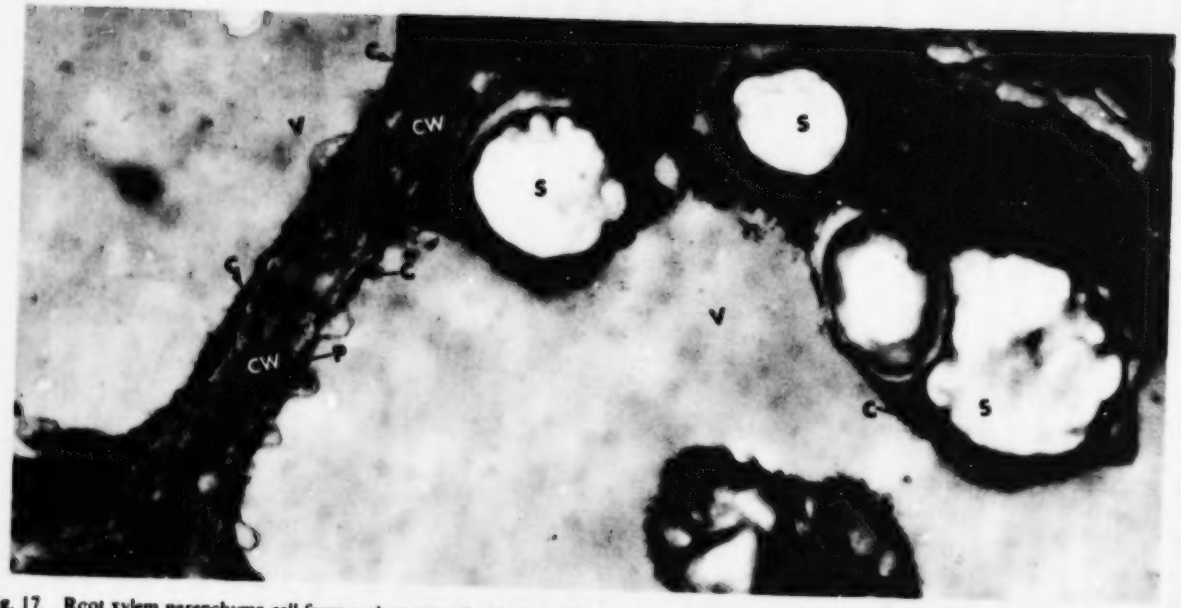

Fig. 17. Root xylem parenchyma cell from a plant treated with 62 μg Cd/litre of nutrient solution. Starch grains (S) were seen in nearly every cell observed at this Cd concentration. Magnification 4,750 ×. (Host cytoplasm C, host cell wall CW, plasmalemma P, vacuole V.)

Cadmium concentration in sand culture

Extraction of the sand culture with 0·01N HCl revealed cadmium at a concentration of 0·42 μg/g of dry sand without the addition of nutrient solution containing cadmium (Table 2). The cadmium concentration increased up to 0·63 μg/g of sand when cadmium was added to the nutrient solution. When the sand culture was extracted with deionised water, cadmium was not detected in sand that had been irrigated with nutrient solution without the element. The concentration of water-soluble cadmium increased in the sand with increasing cadmium in the nutrient solution and a high positive correlation was observed ($r = 0·999$). When the sand cultures were extracted with water before extracting with 0·01N HCl, cadmium concentration was observed to increase from 41 to 57 μg/g of dry sand.

The amount of the element adsorbed to the culture vessels is not known.

DISCUSSION

Endophyte resorption is increased in *Alnus rubra* by the addition of cadmium to the nutrient medium. Endophyte resorption has not been studied extensively and the mechanisms involved in stimulation of the process by cadmium are not known. It has been proposed that the resorption phenomenon might be regulated by neighbouring uninfected host plant cells (Lalonde & Knowles, 1975). Cadmium inhibits respiration and stimulates the activity of several enzymes associated with senescence (Lee *et al.*, 1976). It is possible that cadmium may also inhibit the degradation of substances that stimulate endophyte resorption or increase the rate at which they are released. Conversely, the release of substances which prevent resorption may be decreased.

The appearance of septa in endophyte vesicles preceded resorption of the endophyte (Figs 9 and 10). Numerous septa may thus be an early indication that cadmium is decreasing the nitrogen fixing capacity of *Alnus rubra* nodules. However, cadmium may alter the staining characteristics of endophytes exposed to the element and thus allow already existing septa to become less electron-dense. Although septa were not easily seen in the endophyte vesicles from *Alnus rubra* plants exposed to cadmium, they were observed in normal vesicles from *Alnus crispa* (Lalonde & Knowles, 1975).

Nitrogenase activity decreased while the number of nodules per plant increased (Table 1). The reduced enzyme activity was apparently compensated by an increased number of root nodules. The high negative correlation ($r = -0·96$) observed between nitrogenase activity and cadmium concentration in the nodules suggests that the element directly affects the enzyme system. These data indicate that cadmium selectively inhibited the nitrogen fixation system in the concentration range of 6 to 62 μg/litre of nutrient solution.

The prominent nucleoli in the root cells of *Alnus rubra* treated with cadmium may

be explained in terms of altered structural characteristics that permit dense staining. Increasing prominence of membranes and cell organelles in yeast cells exposed to cadmium has been reported (Lindegren & Lindegren, 1973). Cadmium altered the characteristics of yeast cell membranes, resulting in heavy staining of electron-dense material. There is, however, insufficient information to account for the failure to observe nucleoli in the root cells of control *A. rubra* plants. Studies on the ultrastructure of the roots of nodulated *Alnus* species have not been previously reported.

Nucleoli are indicative of resting cells and are less likely to be observed in rapidly dividing cells. The root dry weight data in Table 1 indicate that root cells exposed to cadmium were dividing more slowly than corresponding control cells. Therefore, nucleoli remained visible longer and were more likely to be observed in cells from plants given cadmium. Starch grains were apparent in the root cells of plants exposed to cadmium, but were not obvious in cells of comparable control plants. Inhibition of cellular growth by cadmium in the roots could lead to the formation of starch. This is based upon the assumption that cellular growth utilises a major part of the carbohydrate reserves in cells not exposed to cadmium. Further research is necessary in order to elucidate the mechanisms by which cadmium influences the ultrastructure of *Alnus rubra* root and nodule cells.

Since there was a statistically insignificant increase in the amount of cadmium adsorbed to the sand particles with increasing cadmium in the nutrient solution, a large proportion of the element may have been adsorbed to the culture and/or receiving vessels. Water extraction of the sand indicated that the water-soluble cadmium was the portion of the element which affected the plant physiological processes. The ions in solution are available for plant uptake by roots and translocation to the other organs. The water-soluble cadmium accounted for 2·3 to 22% of the element extracted from the sand and the percentage increased as the concentration of cadmium in the nutrient solution increased from 6 to 62 μg/litre (r = 0·99) (Table 2). The measurements associated with response of *Alnus rubra* plants to cadmium (Table 1) varied with the concentration of plant-available cadmium in the sand. The concentration of plant-available cadmium was decreased by adsorption to the sand and culture vessels. Lowering the pH of the nutrient solution to a more acid value would probably have increased cadmium uptake.

The cadmium concentration of 11 μg/g observed in the tops of plants from the *Alnus* stand may not reflect the concentration of the element in the plant tissue since cadmium may be adhering to the surface of the leaves and stems. However, the roots were cleaned thoroughly with deionised water to remove all traces of soil before analysing for cadmium. Because of the thorough rinsing, the element may have been partially leached from the intercellular spaces of the outer cell layers. This could result in a concentration of cadmium in roots (11 μg/g) that is lower than the actual concentration. Thus, it is difficult to compare *Alnus rubra* plants in the forest with those in the greenhouse, although harmful levels of cadmium appear to accumulate

in the tops of both groups of plants. The plants from the *Alnus* stand were approximately the same age and size as those used in the greenhouse study; therefore, cadmium concentrations in plants grown in the forest may approach those found in the greenhouse at the same stage of development. Cadmium concentrations ranging from 103 to 765 ppm have been observed in leaf specimens taken from sampling sites around a lead smelter (Gale & Wixson, 1979).

Observations from this experiment indicate that cadmium decreases the nitrogenase activity—and therefore nitrogen fixation—in *Alnus rubra* by stimulating endophyte resorption in the nodules. Nitrogenase activity has been associated with the vesicles of *Alnus* species (van Straten *et al.*, 1977). Therefore, loss of vesicles would result in loss of nitrogen-fixing capability. The observations also indicate that cadmium reduces the rate of respiration in root cells since starch, a carbohydrate reserve, accumulated in cells exposed to the element.

ACKNOWLEDGEMENTS

We wish to thank Messrs R. B. Addison and A. H. Soldner for assistance in electron microscopy, Dr F. R. Rickson for assistance in the interpretation of the electronmicrographs and Z. Toohill for preparing the manuscript.

REFERENCES

ALLAWAY, W. H. (1968). Agronomic controls over the environmental cycling of trace elements. In *Advances in agronomy*, Vol. 20, edited by A. G. Norman, 235–74. New York, Academic Press.

FISHBECK, K., EVANS, H. J. & BOERSMA, L. L. (1973). Measurement of nitrogenase activity of intact legume symbionts *in situ* using the acetylene reduction assay. *Agron. J.*, **65**, 429–33.

GALE, N. L. & WIXSON, B. G. (1979). Cadmium in forest ecosystems around lead smelters in Missouri. *Environ. Hlth Perspectives*, **28**, 23–7.

HUANG, CHI-YING, BAZZAZ, F. A. & VANDERHOFF, L. N. (1974). The inhibition of soybean metabolism by cadmium and lead. *Pl. Physiol., Lancaster*, **54**, 122–4.

LALONDE, M. & KNOWLES, R. (1975). Ultrastructure of the *Alnus crispa* var. *mollis* Fern. root nodule endophyte. *Can. J. Microbiol.*, **21**, 1058–80.

LEE, K. C., CUNNINGHAM, B. A., PAULSEN, G. M., LIANG, G. H. & MOORE, R. B. (1976). Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. *Physiologia Pl.*, **36**, 4–6.

LINDEGREN, C. C. & LINDEGREN, G. (1973). Mitochondrial modification and respiratory deficiency in the yeast cell caused by cadmium poisoning. *Mutation Research*, **21**, 315–22.

SPECIAL STUDIES STAFF. (1975). Environmental appraisal. In *Scientific and technical assessment report on cadmium*, Chapter 5, 5-1 to 5-24. U.S.E.P.A. Star Series, EPA-600/6-75-003. U.S. Government Printing Office, Washington, D.C.

TARRANT, R. F. (1961). Stand development and soil fertility in a Douglas fir–red alder plantation. *Forest Sci.*, **7**, 238–46.

TARRANT, R. F. & MILLER, R. E. (1963). Accumulation of organic matter and soil nitrogen beneath a plantation of red alder and Douglas fir. *Proc. Soil Sci. Soc. Am.*, **27**, 231–4.

VAN STRATEN, J., AKKERMANS, A. D. L. & ROELOFSEN, W. (1977). Nitrogenase activity of endophyte suspensions derived from nodules of *Alnus*, *Hippophae*, *Shepherdia* and *Myrica* spp. *Nature, Lond.*, **266**, 257–8.

A SELECTION OF BOOKS PUBLISHED BY APPLIED SCIENCE PUBLISHERS

BIOLOGICAL MONITORING OF INLAND FISHERIES
edited by J. S. Alabaster
6 × 9". xvi + 226 pages. 22 illus.

ECOLOGICAL ASPECTS OF THE TOXICITY TESTING OF OILS AND DISPERSANTS
edited by L. R. Beynon and E. B. Cowell
6¼ × 10". viii + 149 pages. 26 illus.

ECOLOGICAL EFFECTS OF OIL POLLUTION ON LITTORAL COMMUNITIES
edited by E. B. Cowell
6 × 9". vii + 248 pages. 86 illus.

ENVIRONMENTAL IMPACT OF MINING
by C. G. Down and J. Stocks
6 × 9". ix + 371 pages. 108 illus.

ESTUARINE ENVIRONMENT
edited by R. S. K. Barnes and J. Green
6 × 9". xii + 133 pages. 33 illus.

LEAD IN THE ENVIRONMENT
edited by P. Hepple
6 × 9". ix + 82 pages. 7 illus.

MARINE ECOLOGY AND OIL POLLUTION
edited by Jonathan M. Baker
6 × 10". vii + 566 pages. 168 illus.

A SELECTION OF BOOKS PUBLISHED BY APPLIED SCIENCE PUBLISHERS

MOSQUITO ECOLOGY: FIELD SAMPLING METHODS
by M. W. Service
6 × 9". xii + 583 pages. 75 illus.

PETROLEUM AND THE CONTINENTAL SHELF OF NORTH WEST EUROPE: VOLUME 2: ENVIRONMENTAL PROTECTION
edited by H. A. Cole
8¼ × 11¾". vii + 126 pages. 37 illus.

WASTE TREATMENT IN AGRICULTURE
by P. N. Hobson and A. M. Robertson
6 × 9". x + 254 pages. 21 illus.

BIODETERIORATION INVESTIGATION TECHNIQUES
edited by A. Harry Walters
6 × 9". xii + 346 pages. 81 illus.

PROCEEDINGS OF THE 3RD INTERNATIONAL BIODEGRADATION SYMPOSIUM
edited by J. Miles Sharpley and Arthur M. Kaplan
6½ × 10". xiv + 1138 pages. 452 illus.

ANIMAL WASTES
edited by E. Paul Taiganides
6 × 9". xvi + 429 pages. 98 illus.